Original Article

Identification of Group "A & AB" Major Sub-Groups in a Mixed Pakistani Population of Karachi

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ABSTRACT

Objective: To determine the frequency of weaker sub-group A2 and A2B in the catchment area of Dow international medical college, Karachi.

Place & Duration: Observational cross sectional study conducted in Dow diagnostic research and reference lab (DDRRL) blood bank from 1st January 2013 till 30th June 2013.

Material and methods: A total of 1424 healthy unrelated donors and their patients for whom they donated blood, of both sexes and reporting to DDRRL blood bank were selected. Forward and reverse ABO grouping was done and as A and AB blood groups were isolated. These groups were then tested for sub-group A2 and A2B and their numbers were expressed as percentages of their respective blood groups. Results: Amongst 387 A group individuals, n= 362 (93.54 %) were A1 while n=25 (6.46 %) were A2 sub-group. From AB n= 101, n = 85 (83.17 %) belonged to A1B sub-group while n=17 (16.83 %) were A2B sub-group

Conclusion: There is substantive presence of the most frequently occurring subgroups, A1, A2, A1B and A2B in Pakistani population and their frequency is nearly the same as reported in literature

Key words: ABO, A2, Lectin A1, Subgroups, A1, Antibody, Anti-sera, A2B sub-group.

INTRODUCTION

The major blood groups have a Co-dominant mode of inheritance and up till now more than 400 red cell antigens have been discovered. ABO blood group system is the most common and highly significant in transfusion medicine. There are over 70 ABO alleles reported to date highlighting the extensive sequence variation in the coding region of the gene[1].

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A1 antigen is the most common A antigen and commonly known as A group antigen The presence of A2 antigen has produced A2 and A2B subgroups of A and AB groups respectively. In addition there are other more weaker A antigens accounting for a greater diversity of A and AB groups but the most common is the A2 antigen. For many decades, the basis of A1 and A2 phenotypes has been a subject of debate. It is now known that there are different transferases for each specific major blood group encoded by specific blood group alleles to convert the H substance to the specific group[2]. About 1-8% of A2 and 22 % of A2 and A2B individuals have cold anti-A1 antibodies but have the potential to develop antibodies reacting at 37°C, if transfused with A1 or A1B blood, leading to severe hemolytic transfusion reactions in the recipient[3].

The low performance or production of lesser antigenic sites by A2 allele is probably due to mutation in the A2 glycosyltransferase pepti

chain including the common A2 deletion in the coding region, which produces a protein with 21 extra amino acids. It is also a fact that A1 and A2 transferases have different pH optimum, K m values and ion requirements[4].

As time passed by, the possible explanation for the above mentioned quantitative and qualitative differences between A1 and A2 antigens became clearer. A2 phenotype is defined by an allele which encodes for a transferase that is relatively inefficient as compared to the A1 transferase. Due to this allelic difference, A2 and A2B subgroups have lesser number and slightly different structural A antigens on the cell membrane expressing four time sless the number of antigens than A1[5]. The other difference is that A2 subgroup has no or very little converted Ac& Ad branched chains on the surface of red cells due to none or decreased conversion of branched H chains. A type 1& 2 glycolipid (Aa, Ab) structures was found to be present in large amounts in both phenotypes while, type 3 & 4 (Ac& Ad) glycolipid structure was nearly undetectable in the A2 phenotype. With the use of purified glycolipids and specific monoclonal antibodies, it was shown that the major glycolipid difference between A1 and A2 phenotypes is the dominance of "A" type 4 glycolipid in the A1 phenotype[6].

There are four different forms of "H" antigens, two of which, H1 and H2 are un-branched straight chains while the remaining two H3 & H4 are complex branched chains. All these are H antigens and on all of them "A" transferases acts to convert into blood group "A" active glycolipids[6].

The transferases for A1 and A2 are different qualitatively[7]. A1 transferase is efficiently able to convert all the H chains into Aa Ab, Ac& Ad so that no H chains are left unchanged. A2 transferase on the other hand although able to convert un-branched H chains to Aa& Ab but fails to do in case of complex branched H chains, so that there is no formation of Ac& Ad . So the cells of A2 group lack Ac& Ad while containing a lot of branched chains of unconverted H substance. The absence of Ac and Ad antigens on cells of A2 individuals may lead them more prone to form A1 antibody against Anti-A1 cells harboring Ac & Ad chains. As the A2 individuals have unconverted H antigen on their surface, so they react with Anti-H anti-sera. One point of worth mentioning is the occurrence of anti A1 antibody in a higher percentage (22—35 %) in A2B group as compared to A2 group (1-8%)[8].

The explanation for this fact is rather simple in the sense by appreciating that B transferase is more efficient in converting H substance to B antigen than A1 transferase and converts all four H antigen branches, while A2 transferase is inefficient in conversion of H chains into "A" antigens especially Ac& Ad, as a result no Ac& Ad chains are present leading in a higher frequency of anti-A1 (anti-Ac & Ad) production.

MATERIAL AND METHODS

1424 samples were collected, each from individual recipient and donor including a record of their ethnic group. This mixed population was dominated by Sindhi and Urdu speaking people while frequencies of Pashtoon & Punjabi groups were significantly less while Balochi was negligible Fig 1.1 From each individual whole blood sample was taken from an accessible superficial vein by vacutainer in EDTA tube for cell suspension and plain tube clotted sample for serum. The sample reached the blood bank within half an hour in cool box.

From the EDTA sample, washed 3 % suspensions of red cells were made after three washes in normal saline and the clotted sample was centrifuged at a speed of 150g for serum extraction. Six plain tubes of 75 x 10 mm size, labeled as A, B, AB, D, Auto-control and A1 lectin (in case of A or AB group) were used for cell or forward group. Three glass tubes of same size were labeled as a,b an O for reverse or serum grouping. One drop of each respective anti-serum from BioRad was added in the respective tubes along with the cell suspension of the test sample. For reverse grouping one drop of known cells (a, b and O) 3 % suspension was added along with two drops of test serum. All the tubes were gently mixed and then centrifuged at a speed of 150 g for 21 seconds. The tubes were read for visible agglutination by naked eye, magnifying glass mirror and microscopically.

The positive reactions were noted from 4+ to 1+. Negative results were examined by mirror and under microscope for any agglutination and

RESULTS

Table 1.1 & Fig 1.2, shows the split up of the ethnic population, in the study of a total sample size of 1424 Sindhi n= 580 (41.28 %), Urdu speaking n= 544 (38.7 %), Pathan n= 191 (13.59 %), Punjabi n= 90 (6.4 %) and Balochi n = 19 (1.33 %) were present.

Fig 1.1 From the total people reporting to DDRRL blood bank, n = 1424, B group was the largest one, comprising n= 530 (37.2 %), O group n= 403 (28.3 %), A n= 389 (27.4 %) while AB n= 102 (7.1%) was the least group counted.

From "A" group, A1 n= 364 (93.54 %) & A2 was n= 25 (6.46 %). Table 1.3, shows the frequency of A2 sub-group in Sindhi, Urdu speaking, Pashtoon, Punjabi and Balochi groups was 5.85 %, 6.5 %, 5.80 % , 12 % and nil respectively, while frequency of A2B subgroup in the same groups was 6.93 %, 5.94 %, 2.97 % .99 % and nil respectively.

Table 1.3 Shows the frequencies of A, B, O and AB blood groups in sindhi group as 39.5 %, 13.76 %, 13.61 % and 2.7 % respectively while in Urdu speaking group the frequencies were 35.7 %, 16.92 %, 8.5 % and 3 %. The frequencies of same groups in Pashtoon group were 36.12 %, 27.74 %, 29.84 %, 6.28 % respectively while in Punjabi group were 27.7 %, 35.55 %, 30 % and 6.66 % respectively.

The cumulative frequencies of A2 and A2B, in the whole of studied population were 6.46 % and 16.83 % respectively.

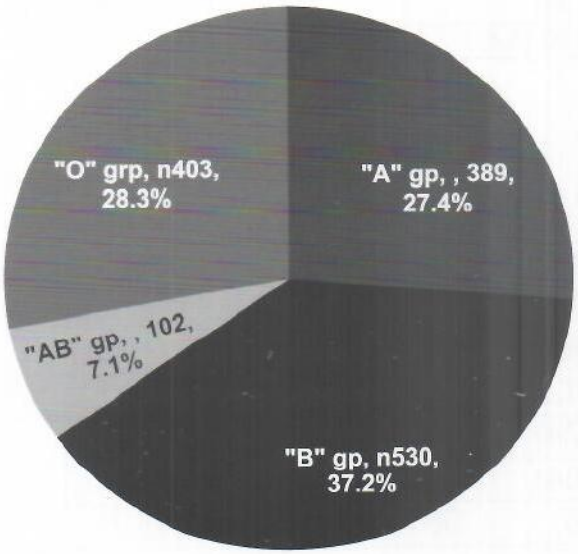

Fig 1.1 Frequencies of Major blood groups

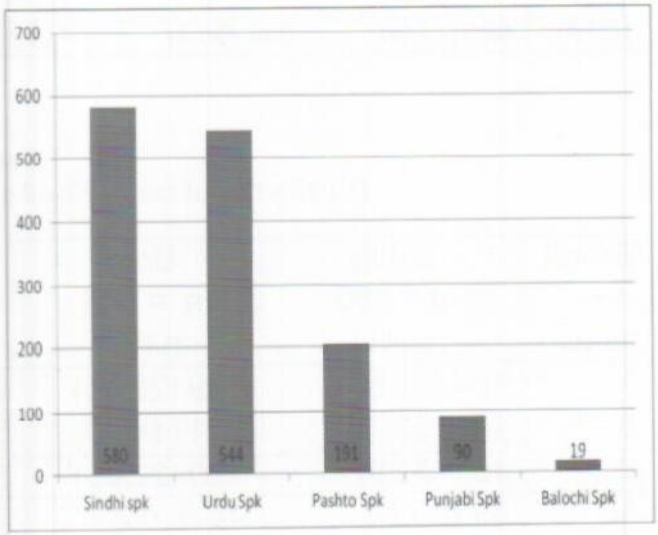

Fig 1.2 Ethnic split up of total sample n 1424

Table 1.2. Split up of A and AB subgroups from specific group.

	A		AB	
	A1 %	A2 %	A1B %	A2B %
Sindhi	94.15	5.85	82.07	6.93
Urdu	93.52	6.5	86.36	5.94
Pashtoon	94.20	5.80	75	2.97
Punjabi	88	12	83.3	0.99
Baluchi	2	0	1	0

Table 1.1.
Complete fractionation of blood groups according to ethnicity: Balochi population is mentioned but not included as they had very small number

Blood Groups	Sindhi n (%) of total	Urdu Speaking n (%) of total	Pashtoon n (%) total	Punjabi n (%) of total	Balochi n (%) of total	Total n (%) of Total studied population
A	154 (39.5)	139 (35.7)	69 (17.7)	25 (6.4) %	2 (0.5)	389 (27.3)
A1	145 (94.15) of A	130 (93.5) of A	65 (94.2) of A	22 (88) of A	2 (0.51) of A	364 (93.57) of A
A2	9 (5.85) of A	9 (6.5) of A	4 (5.80) of A	3 (12) of A	0 (0) % of A	25 (6.42) of A
B	196 (13.76)	241 (6.92)	53 (3.7)	32 (2.24)	8 (0.57)	580 (40.7)
O	191 (13.61)	120 (8.5)	57 (4) of all	27 (1.9)	8 (0.5)	403 (28.3)
AB	39 (2.7)	44 (3)	12 (0.8) of all	06 (0.4)	1 (07)	102 (7)
A1B	32 (82.07) of AB	38 (86.36) of AB	9 (75) of AB	5 (83.3) of AB	1 (0.98) of AB	86 (85.175) of AB
A2B	07 (6.93) of AB	6 (5.94) of AB	3 (2.97) of AB	1 (0.99) of AB	0	17 (16.83)of AB
TOTAL	580 (41.28)	544 (38.7)	191(13.59)	90 (6.4)	19 (1.33)	1424

Table 1.3
Distribution of major blood groups in each Ethnic population

Blood groups	Sindhi n = 580 n (%)	Urdu n = 544 n (%)	Pashtoon n = 191 n (%)	Punjabi n = 90 n (%)	Balochi n = 19 n (%)
A	154 (26.55)	139 (25.55)	69 (36.12)	25 (27.77)	02 (10.5)
B	196 (33.79)	241 (44.30)	53 (27.74)	32 (35.55)	08 (42)
O	191 (32.9)	120 (22.05)	57 (29.84)	27 (30)	08 (42)
AB	39 (6.72)	44 (8.08)	12 (6.28)	06 (6.66)	01 (5.2)

DISCUSSION

It is important to have an accurate idea of blood group frequency in a community in order to plan for the transfusion requirements in medical care. This aspect can be further emphasized by detecting the percentage of important sub-group A2 and A2B of group A and AB respectively. This subgroup becomes significantly important in transfusion medicine, stem cell and organ transplant, ABO HDNB and in blood compatibility testing. Its importance is further escalated by the naturally occurring Anti-A1, which is usually a cold antibody and no significance during routine transfusion practice but can be a problem if it develops anti A1 antibody acting at 37 C with a possibility of severe hemolytic transfusion reaction if A1 blood is transfused to A2 individual harboring anti A1[9].

There is no doubt that quite a number of studies have been done in Pakistan regarding the occurrence of major blood groups, but the literature is sparse in the context of the occurrence of A2 and A2B sub-groups. This study was able to detect the different blood group frequency of major blood groups including A2 sub-group in a mixed Pakistani population of Karachi.

It was a great development in medical practice when ABO blood group system was discovered about a century ago. The second significant step was the identification of sub-groups of ABO system by von Dungeon, especially A2 group. Categorization into A1 and A2 phenotypes accounts for 99% of all cases of "A" group individuals. The cells of approximately 80% of all group A or AB individuals are A1or A1B, while the remaining 20% are A2 or A2B or weaker subgroups[10]. The present study is a relatively first comprehensive study that documents the frequencies of A2 and A2B sub-groups of "A" and "AB" blood groups among a mixed population of Karachi.

Another interesting feature of this study is that we did not detect any anti-A1 antibody in the A2 and A2B sub-groups, which is in contrast to international literature where 1—8 % of A2 groups and 22-35 % of A2B contains anti A1 and anti A1B antibodies respectively. This study could be very helpful for meeting the blood requirement in Karachi especially for our local blood and central blood bank, where the requirement of certain blood groups may be more than others especially in situations of emergency or national disasters. This study could also be helpful in the emphasizing the importance of both cell and serum grouping to certain centers where only cell or forward grouping is done, as preformed antibodies against A1 group can be easily missed.

The fact that bone marrow transplant has emerged as a curative therapy to a large extent in hereditary blood diseases and quite significant cases of malignant hematology, so it is of prudent importance that if recipient is A2 group, it is preferable to detect anti-A1 antibodies, if "A" group blood products are given and if not then one should anticipate the problems of transfusing A1blood group. Further studies are needed to identify other weaker sub-groups of A and B in order to get a good idea of their occurrence and frequencies in Pakistan.

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